Characterisation of the cartilage/bone interface utilising reflectance spectroscopy

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Abstract-**Optical reflection spectra of the cartilage/bone interface from hip joints of cows were studied. When comparing to ultrasonic measurement, it was found that cartilage thickness could be extracted using optical reflectance spectroscopy. For thicker cartilage layers, a high reflection for the wavelengths 400-600 was seen, and for thinner cartilage layers, the characteristic spectra of blood and bone dominated. The optical reflectance spectra may be used to characterise cartilage, and specifically cartilage thickness, in connection with in situ diagnosis or autologous chondrocyte implantation (ACI).**
Keywords-**Cartilage, reflection spectroscopy, ultrasound, autologous chondrocyte implantation**

I. Introduction

Researchers in the field of cartilage and bone repair have looked into the prospects of using autologous therapies and tissue engineering. Autologous therapies have traditionally involved using patient's own cells as a treatment. In the case of autologous chondrocyte implantation (ACI), chondrocytes are taken from biopsies from the patient, grown in cultures *ex vivo* and then replaced as free cells enclosed in a perioseal flap. Successful repair has been demonstrated after a mean follow-up of 57.7 months in 80% of the patients who had a femoral condyle graft [1-2].

In connection with ACI treatment it is important to be able to assess the optical properties of cartilage and spongeous bone *in situ*, before and after treatment. Studies of the optical properties of cartilage has so far been conducted as *in vitro* experiment in double-integrating spheres using the adding–doubling algorithm for calculation of the optical parameters [3]. This method is from obvious reasons unsuitable for clinical investigations. So is ultrasound because of the size of probes required. Thin probes can be manufactured from optical fibres, which can be introduced into the joints and used for reflectance spectroscopy and confocal microscopy. Reflectance spectroscopy can tentatively reveal cartilage surface defects as well as areas of thinner cartilage, necessary to repair, and can thus be a more appropriate method than earlier reported methods [4].

The aim of this work was to evaluate reflectance spectroscopy as a tentative tool for assessment of cartilage/bone properties in connection with *in situ* diagnosis and ACI.

II. Methodology

Five condyles of femur from younger cows were obtained from a local slaughterhouse less than 24 hours after sacrifice. The condyles were cleaned in saline and prepared for cartilage measurements through removal of soft tissues and tendons surrounding the joint. Three sites on each condyle surface were used for the measurements. The condyle was fixed to the end of a metal rod so it could rotate around the axis of the metal rod. A handheld, rotating, grinding machine was used to reduce the cartilage layer thickness. A sandpaper with roughness P100 was used for grinding. Care was taken to grind in short episodes (5-15s) not to increase the temperature of the cartilage.

Measurement of cartilage layer thickness was performed with high-resolution ultrasound (A-scan 20 MHz, Dermascan 3, version 3, Cortex Technology, Hadsund, Denmark). The probe was scanning over the measurement site and an image of the cartilage/bone interface was presented on the computer screen from which the cartilage layer thickness could be measured by using two cursors (Fig. 1). The resolution of the thickness measurement was approximately 0.1 mm.

Optical reflection spectra were recorded by using an Oriel Instaspec IV CCD spectrometer equipped with an Ocean Optics broad spectrum tungsten lamp HL 2000 (spectral range 0.36-2μm). The spectrum was calculated according to the formula:

$$I(\lambda) = \frac{I_{tissue} - I_{background}}{I_{reference} - I_{background}} \quad (1)$$

where

I_{tissue}	is the raw spectrum of the examined tissue
$I_{background}$	is the detector background signal
$I_{reference}$	is the diffuse reflectance spectrum from a white reference ($BaSO_4$)

The spectra were studied before grinding and for decreasing cartilage layer thicknesses. The condyle and the measurement site were documented by means of a digital camera (Nikon CoolPix 990, Japan).

III. Results

Mean reflectance spectra of the material before first grinding and after final grinding are presented in Fig. 2. The mean reflectance spectrum of the excised bone surfaces is also included in the figure. Before grinding, the cartilage thickness was 4.1 ± 1.1 mm (mean ± SD, n = 15) and after final grinding, the corresponding value was 0.3 ± 0.2 mm (n = 15). The reflectance at lower wavelengths seems to

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Abstract

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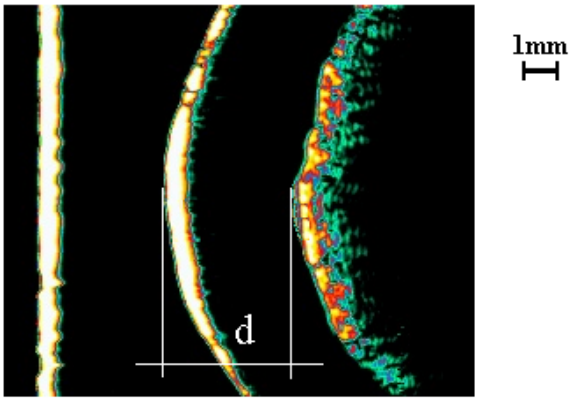

Fig. 1. Ultrasound image of the cartilage/bone interface including the procedure of reference cartilage thickness (d) determination. The plastic film is covering the ultrasound transducer.

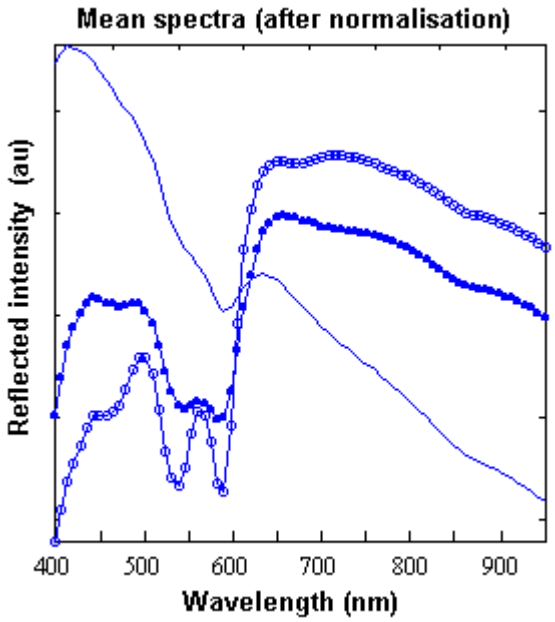

Fig. 2. Mean reflectance spectra before grinding (d = 4.1 ± 1.1 mm, n = 15, solid line), after final grinding (d = 0.3 ± 0.2 mm, n = 15, solid points) and for excised bone (d = 0 mm, n = 5, open circles).

correspond to the cartilage thickness. Furthermore, absorption peaks according to haemoglobin cause characteristic valleys for less cartilaged bone. A typical series of spectra from a grinding session is presented in Fig. 3. Bone spectra from the joint is included in the figure.

IV. Discussion

The main finding of this study is the possibility to extract information on cartilage thickness by studying the reflectance spectrum of the cartilage surface. This would make it possible to use minimally invasive technique to characterise cartilage in connection with *in situ* diagnosis or ACI. The reflectance spectra of cartilaged bone can be seen as a sum of spectra from cartilage, bone and blood. The character of the three spectra can be seen in Fig. 2, showing a high reflection at lower wavelengths for cartilage and bone, and the characteristic valleys of haemoglobin absorption. Our intention is to characterise the cartilage/bone interface by studying the relative content of these three components in the combined reflectance spectra. In this way, blood perfusion of the bone can be estimated and compensated for, whilst the cartilage thickness is seen from the relation between the bone and cartilage components. By using this approach, the accuracy of cartilage thickness determination only becomes dependent upon the variability of these three spectra. This variability remains to be investigated for a larger human material but according to the results of this study, only minor variations are expected.

Methodological issues to consider include the choice of reflectance spectroscopy instead of optical coherence tomography (OCT). OCT is a promising technique for studying layered biological material [5]. Our belief, however, is that there will be difficulties with this technique in this application due to the high scattering and absorption of cartilage and bone, and thus, a low penetration depth [3,6-7].

It is well known that the collagen of cartilage is structured and lined. In preliminary measurement, this effect was found to influence the reflectance spectra, but to what degree this will influence the *in situ* situation is unknown.

V. Conclusion

After studying the cartilage/bone interface from hip joints of cows, it was found that information on cartilage thickness could be extracted using optical reflectance spectroscopy. For thicker cartilage layers, a high reflection for the wavelengths 400-600 nm is seen, and for thinner cartilage layers, the characteristic spectra of blood and bone dominate. Consequently, the optical reflectance spectra may be used to characterise cartilage, and specifically cartilage thickness, in connection with *in situ* diagnosis or ACI.

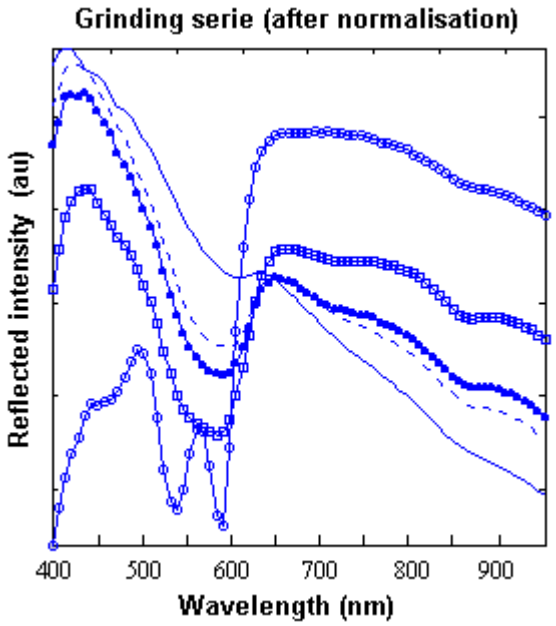

Fig. 3. Reflectance spectra from a grinding session. Before grinding (d = 3.5 mm, solid line), after first grinding (d = 2.6 mm, dashed line), after second grinding (d = 0.9 mm, solid points) and after third (final) grinding (d = 0.4 mm, open squares). Spectrum from excised bone is included (open circles).

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REFERENCES

[1] M. Brittberg, A. Lindahl, A. Nilsson, C. Ohlsson, O. Isaksson, and L. Peterson, "Treatment of deep cartilage defects in the knee with autologous chondrocyte transplantation," *N. Eng. J. Med.*, vol. 331, pp. 889-895, 1994.

[2] L. Petersen, "Autologous chondrotransplantation. Articular cartilage regeneration and transplantation," *65th Annual Symposium, American Academy of Orthopaedic Surgeons*, New Orleans, 1998.

[3] S.A. Prahl, M.J.C. van Gemert, and A.J. Welsh, "Determining the optical properties of turbid media by using the adding-doubling method," *Applied Optics*, vol. 32, pp. 559-568, 1993.

[4] G.A. Athesian, L.J. Soslowsky, and V.C. Mow, "Quantitation of articular surface topography and cartilage thickness in knee joints using stereophotogrammetry," *J. Biomechanics*, vol. 24, pp. 761-776, 1991.

[5] J. Welzel, "Optical coherence tomography in dermatology: a review," *Skin Res. Technol.*, vol. 7, pp. 1-9, 2001.

[6] J.F. Beck, P. Blokland, P. Posthumus, M. Alders, J.W. Pickering, H.J.C.M. Sterenborg, and M.J.C. van Gemert, "In vitro double-integrating-sphere optical properties of tissues between 630 and 1064 nm," *Phys. Med. Biol.*, vol. 42, pp. 2255-2261, 1997.

[7] R. Raunest, and H. Schwarzmaier, "Optical properties of human articular tissue as implication for a selective laser application in arthroscopic surgery," *Lasers Surg. Med.*, vol. 16, pp. 253-261, 1995.